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Phenotypic and Molecular Characterization of *Escherichia coli* O157:H7 Isolates from Dogs (*Canis lupus familiaris*)

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This study aimed to detect and characterise phenotypically and molecularly *Escherichia coli* O157:H7 isolates of canine origin (*Canis lupus familiaris*). Initially, a bank of 122 *E. coli* strains was screened for the O157:H7 serotype, yielding 4 positive samples. Among the total strain bank, the virulence genes eaeA and hlyA were detected in 5.74% (7/122) and 0.82% (2/122) of isolates, respectively, while stx1 and stx2 were not found. To further investigate horizontal gene, transfer and resistance profiles, transconjugation experiments were performed using the O157:H7-positive isolates, resulting in nine transconjugants. PCR analysis of the transconjugants revealed the presence of eaeA (88.88%), hlyA (44.44%), and resistance genes such as blaTEM (77.77%), blaCTX-M (88.88%), and tetA (22.22%). Disk diffusion (Kirby-Bauer) assays confirmed that 88.88% of isolates were phenotypically resistant to ESBL and AmpC antibiotics. Resistance to specific antimicrobials included cefotaxime (55.55%), ciprofloxacin (22.22%), enrofloxacin (33.33%), and sulfamethoxazole/trimethoprim (44.44%). Notably, resistance to ampicillin, vancomycin, and erythromycin was observed in 88.88% of isolates. This study highlights the significant prevalence of multi-drug resistant *E. coli* O157:H7 and the potential for plasmid-mediated gene transfer in canine populations in central Ecuador, raising important concerns for public health.
Keywords: Canine, enterobacteriaceae, shiga toxigenic, gram-negative, enterohemorrhagic, human disease.

INTRODUCTION

Escherichia coli is a member of the Enterobacteriaceae family and is part of the microbiota of the large *intestine of animals and humans* (Vega-Manriquez et al., 2020). The possibility of obtaining genetic components through horizontal transfer led to the emergence of pathogenic strains that have been categorized as intestinal or extraintestinal and are responsible for different clinical conditions (Cundon et al., 2018).

Shiga toxigenic toxin-producing *E. coli* (STEC), along with other strains, are Gram-negative, rod-shaped bacteria. However, STEC are characterized by the production of Shiga toxins (Stx). Depending on the cited reference, there are 200 to 400 STEC serotypes, many of which have not been implicated in human disease. However, a subset of enterohemorrhagic *E. coli* (EHEC), O157:H7, is currently the most predominant strain, accounting for approximately 75% of enterohemorrhagic *E. coli* (EHEC) infections worldwide. Other O157 EHEC serotypes are emerging as a cause of foodborne illness (Tiffer, 2016).To determine the susceptibility of a bacterium to an agent, there are antibacterial sensitivity assessment methods that help veterinarians determine the most appropriate antibiotics for a treatment (Sánchez, 2018). The Clinical and Laboratory Standards Institute (CLSI) provides recommendations for antibacterial agents against different diseases and animal species (Pragya et al., 2020). It is necessary to know how to interpret the results, which can be quantitative or qualitative. The agar diffusion or Kirby-Bauer technique is a qualitative method, and its results can be classified as sensitive, intermediate, or resistant, useful in rapidly growing bacteria. The minimum inhibitory concentration (MIC) is a quantitative method that defines the minimum concentration of antibiotic that, over a period of time, is capable of

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inhibiting the growth of a previously standardized bacterial inoculum (Torkan et al., 2016). Likewise, to determine the presence of resistance genes, the polymerase chain reaction (PCR) is used, which reproduces in vitro the physiological process of DNA duplication in cells, exponentially amplifying a specific sequence of double-stranded DNA. (Muñoz, 2020).

Given that STEC strains are important reservoirs of antimicrobial resistance genes, their presence in canine feces represents a significant public health concern. People with close contact to dogs—including owners, children, and veterinarians—face increased risk of exposure (Vega-Manriquez et al., 2020). In Ecuador, infectious diarrhea is one of the leading causes of mortality (Egas, 2016), underscoring the urgency of characterizing E. coli virulence and resistance mechanisms to inform empirical treatment strategies and mitigate zoonotic transmission risks. Despite this, there is limited information on the prevalence and molecular characteristics of *E. coli* O157:H7 in dogs, particularly in central Ecuador. This study aims to address this gap by detecting and characterizing *E. coli* O157:H7 isolates of canine origin through phenotypic and molecular methods, including an assessment of virulence genes, antimicrobial resistance profiles, and the potential for horizontal gene transfer via transconjugation.

MATERIALS AND METHODS

***Characterization of experimental unit*:** This research is part of the study conducted by (Núñez, 2018) "Determination of the presence of emerging antibiotic resistance genes in *Escherichia coli* isolates from canines in the city of Ambato." For this purpose, 122 samples were taken from the strain bank. The study factors were: identification of serotype O157:H7, virulence genes: stx1, stx2, eae, hlyA, resistance genes: $\boldsymbol{bla_{TEM}}$, $\boldsymbol{bla_{SHV}}$, tetA, sul-1, QnrA, QnrS, $\boldsymbol{bla_{CMY}}$, and phenotypic and genotypic profiles of ESBL and AmpC. Obtaining *E. coli* isolates from canines in the city of Ambato.

***Procedure*:** The strains used in the research (122) were previously obtained from rectal swabs of dogs from veterinary clinics and stray dog shelters.

Preparation of the inoculum

- Using a sterile inoculation loop or needle, a sample of each of the *E. coli* isolates cryopreserved in the strain bank was taken.
- The bacteria were suspended in 5 ml of TSB in a test tube and incubated at 37°C for 24 hours (overnight- O/N).
- Using a micropipette, 0.2 ml of the O/N of the bacteria was taken and placed in a test tube with 5 ml of TSB.
- It was incubated at 37°C for 3 hours, until turbidity was observed at an optical density of 0.5 on the McFarland scale (Silva *et al.*, 2023).

***Storage of bacterial colonies*:** The bacterial colonies that were suspected were preserved through cryopreservation at -20°C. Previously they were inoculated and incubated, as mentioned above, then glycerol was added until reaching a concentration of 30%, at which point 700 µl of the culture was added, plus 300 µl of glycerol. Finally, it was placed and labelled in 1.5 mL cryovial tubes per stock (García & Mario, 2019; Ochoa & Sánchez, 2017).

Extraction of bacterial DNA (thermal shock method): Cryopreserved samples from the microorganism bank were reactivated using a swab and inoculated into a test tube with 5 ml of TSB. They were then incubated at 37°C for 12-16 hours (Over/Narrow (O/N)). The next day, 0.5 ml of the (O/N) was taken, after which it was centrifuged in the MiniSpin brand microcentrifuge, F-45-12-11 rotor, 6 cm radius, Eppendorf manufacturer, at 672 relative centrifugal force (RCF) for 3 minutes. The supernatant was discarded, and the pellet was resuspended in nuclease-free distilled water (sterile). The surface supernatant was collected and heated in a water bath at 100 ° C for 10 minutes and cooled to -20 ° C for 10 minutes. The supernatant served as a DNA sample that was subsequently analyzed by PCR (García & Miranda, 2018; Suárez & Yañez, 2018).

Agarose gel electrophoresis

- The agarose solution (1% TBE IX) was melted in the microwave for 2 minutes. (70 mL).
- The solution was allowed to cool until it reached 50°C.
- The plate containing the agarose gel was sealed with adhesive tape.
- Once the temperature reached 50°C, 7 µl of SYBER® Safe DNA Gel Stain was added, and the gel was then homogenized.
- The agarose solution containing SYBER® Safe DNA Gel Stain was poured into the previously sealed gel holder. The gel wells were then inserted into the gel holder and left to gel for 15 minutes.
- The adhesive tape that had previously sealed the gel holder was removed and placed in the electrophoresis chamber. The wells should be close to the cathode (negative pole, black).
- 1X TBE was added until the agarose gel was well covered.
- 3 µl of molecular weight marker (Lader) was added to the first well.
- Subsequently, 10 µl of the previously prepared PCR reactions were added to the following wells, until all were filled.
- The electrophoresis chamber was covered, and the electrodes were connected to the power supply.
- The power supply was programmed at 120 volts, 400 mAh, and the electrophoresis was allowed to run for 60 minutes (Salgado, 2016).

Visualization of the amplifications

- Once the electrophoresis was completed, we visualized the DNA fragments using ultraviolet light, and a photograph of the image was taken (Caldes, 2018).

Transfer by conjugation

- ESBL resistance determinants of *E. coli* O157:H7 strains were transferred using Azide-resistant *E. coli* J53 as the recipient strain.
- *E coli* J53 and STEC positive bacteria were cultured on Macconkey Agar and were cryopreserved.
- Colonies of both the donor and recipient bacteria were placed in LB broth.
- They were incubated at 37°C O/N for 3 hours, until turbidity was observed at an optical density of 0.5 on the McFarland scale.
- 4 ml of the donor bacteria and 4 ml of the STEC positive bacteria were placed in a test tube.
- It was incubated at 37°C for 18 hours.
- It was reseeded on Mueller-Hinton agar containing 100 mg/ml Azide and 20 mg/ml Cefotixin.
- Each bacterium was plated on Mueller-Hinton+Azide agar to rule out gene transconjugation.
- Bacterium were cryopreserved.
- The phenotype of the cells obtained by transconjugation was evaluated and verified through antibiograms with the placement of antibiotic discs for ESBL and AmpC, thus evaluating the resistance profiles (Millan *et al.,* 2012).

Placement of antibiotic discs

- The discs of the antibiotic panel to be evaluated were placed on the agar surface using sterile anatomical forceps.
- Light pressure was applied to secure the disc to the agar surface, at a recommended distance of more than 15 mm from the plate edge and more than 30 mm between discs, to avoid overlapping the antibiotic diffusion diameters and, consequently, the inhibition zones.
- After placing the discs, the Petri dishes were incubated at 37°C for 24 hours.
- The plates were placed inverted in the incubator to prevent condensed water from falling onto the agar, thus preventing unwanted changes in the environmental conditions and the reading of the zones (Sánchez, 2019).

Panel of antibiotics used

Table 1. Panel of antibiotics used.

Amikacin (AK30)	Amoxicillin /Clavulanic Acid (AMC30)
Gentamicin (CN10)	Ampicillin /Sulbactam (SAM20)
Piperacillin/Tazobactam (TZP110)	Meropenem (MEM10)
Aztreonam (ATM30)	Imipenem (IMP10)
Cefepime (CEP30)	Ceftriaxone (CRO30)
Ceftazidine (CAZ30)	Cefotaxime (CTK30)
Cefoxitin (FOX30)	Ciprofloxacin (CIP5)
Ac. Nalidixic (NA30)	Erythromycin (E15)
Colistin (CT10)	Sulfamethoxazole /Trimethropin (SXT25)
Vancomycin (VA30)	Ampicillin (AMP30)
Enrofloxacin (ENR5)	

Table 2. Selected antibiotics for ESBL.

Antibiotic	Abbreviation	Sensitive (mm)	Interme-diate (mm)	Resistant (mm)
Amoxicillin/ Clavulanic acid	AMC30	>18	14-17	<13
Cefepime	FEP30	>25	19-24	<18
Ceftazidime	CAZ30	>21	18-20	<17

Source: (CLSI, 2020)

Characterization of bacteria with ESBL and Am phenotype

***BLEE*:** This was confirmed by the double-disc synergy test (ESBL confirmatory test—CLSI) using ceftazidine plus clavulanic acid (CAZAMC), cefepime (FEP), and ceftriaxone plus clavulanic acid (CROAMC). The positive result was interpreted as an increase of ≥ 5 mm in the halo of the cephalosporin in combination with clavulanic acid (Galván et al., 2016).

***AmpC*:** Screening for AmpC β-lactamases was performed using the modified Martinez method, using imipenem (IPM) discs (10 µg) as an inducing antibiotic, supplemented with 20 µL of a phenylboronic acid (PFA) solution (Aldrich®, USA). This is considered a reversible competitive inhibitor of AmpC β-lactamases. The measurement of the halo generated by this disc was compared with the inhibition halo of the IPM disc without the supplement; a difference greater than or equal to 3 mm between the inhibition halos was indicative of the presence of an AmpC β-lactamase (Galván et al., 2016).

Variables

***Independent variable*:** Origin of *E. coli* strains from canines.

***Dependent variable*:** Presence or absence of serogroups and virulence genes: O157, O111, *stx1, stx2, eaeA, hlyA* their phenotypic antibiotic resistance profiles: BLEE (Amoxicillin/Clavulanic Acid, Ceftazidime, Ceftriaxone, Cefepime), AmpC (Piperacillin/Tazobactam, Imipenem) and antibiotic resistance genes: bla_{CTX-M}, bla_{SHV}, bla_{TEM}, *sul1, tetA, QnRA, QnRS.*

Research design: When performing a descriptive evaluation of the resistance profiles of a defined microorganism bank, descriptive statistics were applied (Berrazeg et al., 2013).

Response Variables

Seeding on Mueller-Hinton Agar Plates. (MH)

- Each MH agar plate was appropriately labeled (Isolate Reference, plate number, date) for each organism tested.
- A sterile swab was dipped into the inoculum tube.
- The swab was rotated against the side of the tube, applying firm pressure against the inner walls of the tube to remove excess liquid.
- The dry surface of an MH agar plate was inoculated with the swab three times over the entire agar surface. The plate was continuously rotated to ensure better inoculum distribution.
- The plate was edged with the swab to collect excess liquid, and the swab was discarded in a Class II biohazardous waste container.

- Inside the biosafety cabinet II, the plate was left with its lid ajar, allowing the plate to settle at room temperature for 5 to 15 minutes, favoring the surface of the agar plate to dry prior to incubation (Racínes, 2016).

Measurement and reading of inhibition halos of the anti biogram

- After incubation, the zone sizes of the inhibition zones formed were measured using a ruler.
- When measuring the inhibition zone diameters, the measurements were rounded up to the nearest millimeter whenever necessary.
- The size of the zone was recorded on a record sheet, described in Table 3 (Centurion, 2017; Medrano, 2019).

***Interpretation of antibiogram results*:** The sensitivity results were classified into the three categories according to the CLSI tables: sensitive (S), intermediate (I) or resistant (R) (Pragya et al., 2020).

RESULTS AND DISCUSSION

In the present study, *E. coli* isolates were detected and characterized using two procedures: phenotypic and molecular. The results are presented in two sections. First, from the 122 samples, we identified the presence of *E. coli* O157:H7 and the virulence genes by PCR. Second, from the positive samples of *E. coli* O157:H7, the transconjugation potential with the positive samples was evaluated. Their transconjugates and *E. coli* J53 were tested by PCR, and antibiotic resistance profiles were detected by culture.

***Identify the presence of E. coli serotype O157:H7*:** The 122 samples (Annex 12) were subjected to PCR and the results are shown in Table 6.

Table 3. *Escherichia coli* isolates positive for serotype O157:H7.

Serotype	Statistic	Sample size	Positives	% positives
O157:H7	Clopper-Pearson	122	4	3.28

of the 122 bacteria positive for *E. coli*, 4 (3.28%) were positive for the O157:H7 serotype. This result is inferior because the presence of this serotype is not common in our country and the sample itself is not as large compared to other studies. As reported by Vásquez (2019) who found that out of 104 *E. coli* samples, "18 were positive for O157:H7, 11 out of 16 diarrheal animals were positive, 8 were positive in the case of puppies and 3 in adults. Meanwhile, out of 88 non-diarrheic animals, 7 were positive" and Vásquez (2019) who mention that out of "72 samples, 7 were positive for O157:H7. On the other hand, Amadi et al. (2015) and Vásquez (2019) mention in their research that no isolate was positive for O157:H7, confirming that dogs are not currently reservoirs of the serotype.

They affirm that the diet of the animals studied (canines and felines) mainly consisted of balanced foods, and this circumstance can be considered one of the determining factors for the failure to isolate the microorganism sought in the samples. The consumption of contaminated foods, such as ground meat and raw or undercooked meat products, constitutes one of the main sources of infection for this microorganism.

***Identification of shigatoxigenic genes (stx1, stx2), intimin genes (eae), α-hemolysin secretion gene (hlyA)) by PCR*:** None of the 122 isolates were PCR-positive for the stx1 and stx2 genes, which are responsible for inhibiting protein synthesis within host cells. Ultimately, the death of the host (Melebari, 2023) could have been influenced by the sample size and the incidence of the disease in the region. While 5.74% (7/122) of the isolates were positive for eaeA, causing close adherence of bacteria to enterocytes and destruction of microvilli in the host (Oderiz et al., 2018), 0.82% (2/122) were positive for hlyA, which attacks blood cell membranes, causing cell rupture (Melebari, 2023).

Table 4. *E. coli* isolates positive for the different genes.

Genes	No. samples	No. positive samples	% positive samples
eaeA	122	7	5.74
hlyA	122	2	0.82
stx1	122	0	0.00
stx2	122	0	0.00

These results were because the samples came from both healthy and diseased dogs (Distemper, Trachebronchitis, Gastroenteritis, Neoplasia, Dermatitis, Osteoarthritis, Rickets, Skin Problems, Intussusception, Parvovirus). Confirming that the positive samples belonged to two genes from healthy dogs and seven genes from sick dogs (Parvovirus Gastroenteritis), meaning that dogs without any symptoms can harbor these virulence genes, which in the latter case can cause death. The main source of infection is the culture of our society, since in our country, most people feed their pets raw or mixed BARF diets, which could be the source of the infection. In addition, in most homes, pet owners usually give them milk mixed with kibble or cheese as a treat or snack, foods that Bedasa et al. (2018) confirm the aforementioned by reporting that "in 200 samples examined, the highest isolation of *E. coli* O157:H7 was in raw milk (12%), followed by cheese (5.71%) and meat (3.07%).". In the worst-case scenarios, homes located near a slaughterhouse or tannery are ideal locations for creating another type of transmission, confirming that both forms of transmission are foodborne. According to Bergaglio (2020), the main source of transmission to humans is through the consumption of contaminated food and contact with companion animals, considered emerging modes of transmission.

***Identification of resistance and virulence genes from positive samples of E. coli O157:H7 and its transconjugates*:** Transconjugation potential was evaluated using azide-resistant *E. coli* J53 as the recipient strain.

Table 5. Resistance and virulence genes.

Gen	N° *E. coli* transconjugants analyzed	No. positives	% positives by Gen
bla$_{TEM}$	9	7	77.77
bla$_{CTX-M}$	9	8	88.88
eaeA	9	8	88.88
hlyA	9	4	44.44
tet A	9	2	22.22
bla$_{SHV}$	9	0	0
sul1	9	0	0
QnRA/QnRS	9	0	0
bla$_{CMY}$	9	0	0

From the positive isolates *E. coli* O157:H7 with their respective transconjugates and the J53 bacteria, 9 isolates were found for the blaSHV resistance genes, which is an enzyme capable of hydrolyzing broad-spectrum cephalosporins (Gundran et al., 2019). *sul 1* which is responsible for encoding mutant forms of the target enzyme (Sulfamethoxazole) (Oueslati et al., 2023). *QnRA/QnRS* They are genes that, through the plasmid, mediate resistance to quinolones and *bla$_{CMY}$* As they belong to the group of beta-lactamases, they are characterized by hydrolyzing the amide bond of the beta-lactam nucleus, thus inactivating the antibiotic (Gundran et al., 2019). They were negative because in clinical practice these medications are used as a last option or the therapeutic time is respected. Marcos-Crabajal et al. (2020), obtained different results as they did not find the presence of any of the resistance genes they evaluated, *bla$_{TEM}$*, *bla$_{CTX-M}$*, *bla$_{SHV}$* because in their study the population size was very small. On the other hand, the gene *bla$_{TEM}$* with a percentage of 77.77% (7/9) positives confirming that in our country the correct use of ethical therapy is the main disadvantage when treating diseases or the irresponsibility of pet owners who do not complete the treatment or extend its duration, thus causing the presence of this gene in both sick and healthy dogs. These results are similar to those of Marcos-Crabajal et al. (2020), who identified the *bla$_{TEM}$* gene in 31.4% and the *bla$_{CTX-M}$* gene in 18.6%, indicating that there may be a possible influence of external factors on the presentation of bacterial resistance. That is to say, they were treated at some point in their lives with beta-lactams, which was confirmed by Herrera et al. (2018), who mentions that the production of β-lactamases is the main resistance mechanism in enterobacteria. Most of the responsible enzymes belong to the *bla$_{TEM}$*, *bla$_{CTX-M}$*, *bla$_{SHV}$* families, showing a high result reaffirming what the author mentions. In the case of bla$_{CTX-M}$ and *eaeA*, positive results were obtained in most samples 88.88% (8/9) confirming the above. In the case of *bla$_{CTX-M}$*, and *eaeA* gene, Nimnoi and Pongsilp (2022) mentions that this gene is present in strains of the most virulent serotypes such as O157:H7, O26:H11, thus confirming our results in (Table 8) where the positive isolates of E. coli O157:H7 are found. Sarmiento et al. (2013) mentions that the eaeA gene was found in 12 of 68 (17.6%) isolated from dogs with diarrhea, and 5 out of 76 (6.6%) from dogs without diarrhea demonstrating that a greater number of dogs have this gene. Finally, 22.22% (2/9) tested positive for *tet A* being responsible for causing resistance to Tetracycline which is often transmitted by mobilizable genetic elements, such as plasmids or mobile ones such as transposons. Plasmids harboring tetracycline resistance genes may also carry other antibiotic resistance genes (Sun et al., 2013) for this reason, in the present study we obtained positive results from different resistance genes due to the type of transmission that some genes possess. On the other hand, in most cases, tetracycline is used indiscriminately in veterinary medicine, leading to resistance. Although this gene was found in only two cases, it is worth mentioning that the samples came from healthy dogs, confirming the research conducted by Mejia et al. (2017), who stated that tetracycline resistance was not uncommon. She also mentions that the children were healthy and that none of them had been previously medicated, confirming that the resistant strains appeared to persist well in the microbiota. Her study concluded that in some cases, the babies harbored tetracycline-resistant *E. coli.*

Presence of resistance genes from positive isolates O157, transconjugates and *E. coli* J53 by PCR (Table 6). Detection of antibiotic resistance profiles evaluated through antibiogram (Table 7) . Positive *E. coli* O157:H7 isolates, their transconjugates, and *E. coli* J53 bacteria were tested for 15 antibiotics, with 88.88% resistance to erythromycin, vancomycin, and ampicillin detected in most cases. This is due to the indiscriminate use of antibiotics or their over-the-counter

Table 6. Presence of resistance and virulence genes identified by PCR.

Resistance genes	J53	TR1	TR2	TR3	TR4	CVSP17Sa	CVSP17Sb	CVSP18Ea	CVSP15Sa
bla$_{TEM}$	-	+	+	+	+	+	+	+	-
bla$_{CTX-M}$	-	+	+	+	+	+	+	+	+
bla$_{SHV}$	-	-	-	-	-	-	-	-	-
eaeA	-	+	+	+	+	+	+	+	+
hlyA	-	-	-	+	+	-	-	+	+
sul 1	-	-	-	-	-	-	-	-	-
tet A	-	-	-	-	-	+	+	-	-
QnRA/ QnRS	-	-	-	-	-	-	-	-	-
bla$_{CMY}$	-	-	-	-	-	-	-	-	-

Table 7. Number of *E. coli* O157:H7 strains resistant to the antibiotic panel.

Antibiotic	Resistant No.	%	Intermediate No.	%	Sensitive No.	%	Total samples	Total%
Gentamicin					9	100.00	9	100
Aztreonam			8	88.88	1	11.11	9	100
Cefotaxime	5	55.55	4	44.44			9	100
Cefoxitin					9	100.00	9	100
Ciprofloxacin	2	22.22	6	66.66	1	11.11	9	100
Meropenem					9	100.00	9	100
Amikacin			1	11.11	8	88.88	9	100
Colistin			8	88.88	1	11.11	9	100
Enrofloxacin	3	33.33	1	11.11	5	55.55	9	100
Nalidixic Acid	1	11.11	3	11.11	5	55.55	9	100
Sulfamethoxazole /Trimethoxazole	4	44.44			5	55.55	9	100
Erythromycin	8	88.88			1	11.11	9	100
Ampicillin/Sulbactam			8	88.88	1	11.11	9	100
Ampicillin	8	88.88			1	11.11	9	100
Vancomycin	8	88.88			1	11.11	9	100

sale in agricultural stores to all types of unlicensed individuals. Silva et al. (2023) obtained similar results evaluating cefotaxime (55.55%), ciprofloxacin (22.22%), enrofloxacin (33.33%), nalidixic acid (11.11%), sulfamethoxazole /trimethromycin (44.44%), erythromycin, ampicillin, and vancomycin (88.88%). They also mentioned that resistance to the antibiotics tested can be attributed to the indiscriminate, empirical use of medications, requiring a prior laboratory diagnosis (antibiogram) as a primary factor.

On the other hand, for cefotaxime, 55.55% (5/9) of the samples were resistant. This result is because this medication is used as a broad-spectrum antibiotic in the daily practice of some professionals. Results close to Cuevas-Bravo (2020), who in his research showed that of a total of 34 *E. coli* strains identified, 94% are resistant to cefotaxime. Highlighting that the strains isolated from dogs are more resistant than those from humans due to the exposure to drugs they have through their diet, habits, and coexistence, in addition to the indiscriminate use of drugs by their owners. However, it was found that in the 9 isolates, gentamicin, cefoxitin, and meropenem reflected sensitivity to these antibiotics and amikacin. Therefore, the emergence of Enterobacteriaceae resistant to the wide variety of clinically important antimicrobials is a reality of global interest. For this reason, we suggest identifying antibiotic resistance before prescribing a treatment. This is obtained through cultures and antibiograms.

Identification of bacteria with ESBL phenotype: To determine if the bacteria are ESBL and AmpC, transconjugation tests were performed on the 4 positives of the O157:H7 serotype where there was a recipient bacteria (*E. coli* J53) and donor (each positive sample), with their respective transconjugates obtaining a result of 88%. Similar results with respect to ESBL were obtained by Nimnoi and Neelawan (2022), who, from 30 samples from broiler chickens, obtained 24 positives for ESBL, that is, 80%, while 20% (6/30) obtained the AmpC phenotype, concluding that there is a selective pressure for the appearance of strains resistant to antibiotics, given the poor control and indiscriminate use of them.

Table 8. *E. coli* O157:H7 isolates positive for the different genes.

Sample	Origin	BLEE	AmpC	Azide resistance
CVSP17Sa	CVSP	+	+	
CVSP17Sb	CVSP	+	+	
CVSP18Ea	CVSP	+	+	
CVSP15Sa	CVSP	+	+	
J53	Laboratory	-	-	+
TR1	CVSP17Sa+*E. coli* J53	+	+	+
TR2	CVSP17Sb+*E. coli* J53	+	+	+
TR3	CVSP18Ea+*E. coli* J53	+	+	+
TR4	CVSP15Sa+*E. coli* J53	+	+	+

TR*: (Transconjugate *E. coli* J53+ each bacteria)
,CVSP*: (Santa Prisca Veterinary Clinic)

On the other hand, Nimnoi and Neelawan (2022) evaluated 50 samples from the laboratory of the National University of San Marcos, finding values different from ours. In the case of ESBL, only 2% presented the phenotype, mentioning that its presence compromises the efficacy of treatment with 3rd-generation cephalosporins, and in the case of AmpC, the same was found, 2% stating that despite the result being low, it is alarming to find this type of phenotype since they cause therapeutic failures. With respect to the evaluation of sodium azide, the investigation found transconjugates and *E. coli* J53 positive for ESBL and AmpC, concluding that there was a transfer of resistance genes, confirming that these genes are encoded within plasmids. The discovery of this type of enzyme is of significant clinical importance, even when associated resistance mechanisms have been found. For these reasons, the World Health Organization (WHO) and the World Organization for Animal Health (OIE) promote the

monitoring of multidrug-resistant microorganisms, *E. coli* being of importance.

***Conclusion*:** This study demonstrates the presence of *Escherichia coli* O157:H7 in canine fecal samples from the central region of Ecuador, highlighting a prevalence of 3.28% and the detection of key virulence genes such as eaeA and hlyA. Notably, transconjugation assays confirmed the horizontal transfer of resistance and virulence genes to *E. coli* J53, revealing a high frequency of genes associated with extended-spectrum β-lactamase (ESBL) production (blaTEM, blaCTX-M) and tetracycline resistance (tetA). Phenotypic resistance profiles showed alarming levels of resistance to critically important antimicrobials, including cefotaxime, erythromycin, and vancomycin, underscoring the misuse of antibiotics in veterinary contexts. The presence of ESBL and AmpC phenotypes in both original isolates and transconjugants confirms the dissemination potential of multidrug-resistant *E. coli* strains. These findings emphasize the zoonotic risk posed by companion animals as reservoirs of antimicrobial resistance, and the urgent need for coordinated surveillance, responsible antimicrobial use, and stricter public health policies to mitigate the spread of resistant pathogens.

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